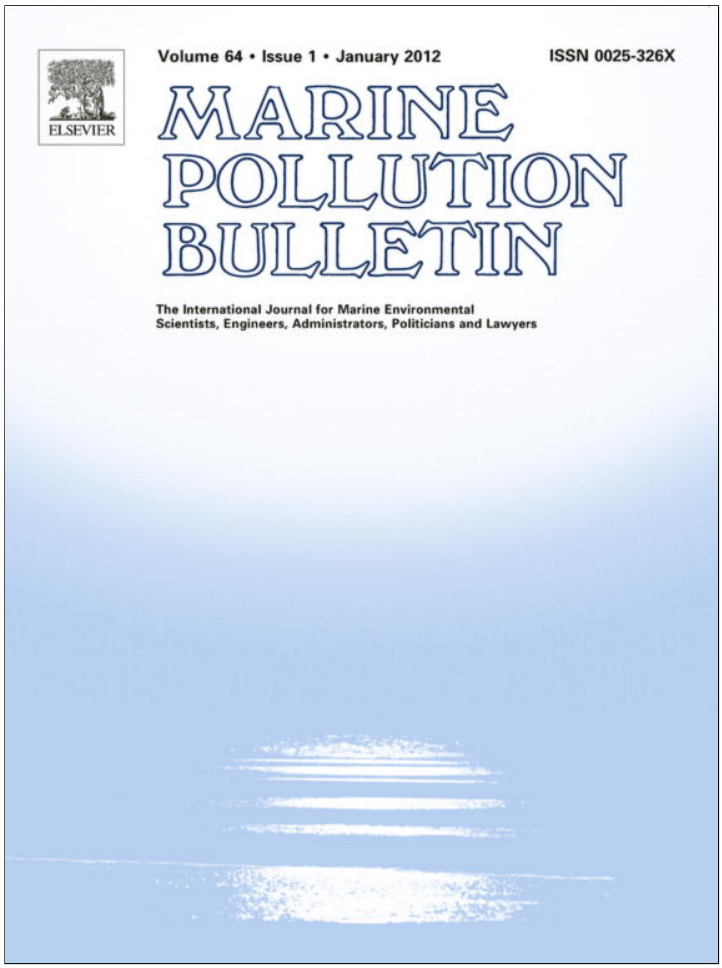

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Assessment of perfluorinated compounds (PFCs) in plasma of bottlenose dolphins from two southeast US estuarine areas: Relationship with age, sex and geographic locations

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ABSTRACT

Plasma PFCs were measured in 157 bottlenose dolphins (*Tursiops truncatus*) sampled from two US southeast Atlantic sites (Charleston (CHS), SC and Indian River Lagoon (IRL), FL) during 2003–2005. ∑PFCs, perfluoroalkyl carboxylates (∑PFCAs), perfluoroalkyl sulfonates (∑PFSAs) and individual compounds were significantly higher in CHS dolphins for all age/sex categories compared to IRL dolphins. Highest ∑PFCs concentrations occurred in CHS juvenile dolphins (2340 ng/g w.w.); significantly higher than found in adults (1570 ng/g w.w. males; 1330 ng/g w.w. females). ∑PFCAs were much greater in CHS dolphins (~21%) compared to IRL dolphins (~7%); ∑PFSAs were 79% in CHS dolphins versus 93% in IRL dolphins. PFOS, the dominant compound, averaged 72% and 84%, respectively, in CHS and IRL dolphins. Decreasing PFC levels occurred with age on the bioaccumulation of PFCs in both sites. These observations suggest PFC accumulation in these two dolphin populations are influenced by site-specific exposures with significantly higher levels in CHS dolphins.

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1. Introduction

Perfluorinated compounds (PFCs) have received increased concern due to their persistence, bioaccumulation and global distribution (Giesy and Kannan, 2001; Houde et al., 2006b, 2011). PFCs are a class of synthetic compounds characterized by chains of carbon atoms of varying length to which fluorine atoms are strongly bonded. They have been widely used as surface coatings and protectants due to their unique surfactant properties, both hydro- and lipophilic, that enhance water, grease and soil repellency (ASTDR, 2009; Kissa, 2001; Lehmler, 2005; Rayne and Forest, 2009). The chemical structure of PFCs makes them extremely stable, resistant to biodegradation, photooxidation, and hydrolysis.

PFCs have been globally detected in surface coastal and ocean waters (Ahrens et al., 2009a; Yamashita et al., 2004) in a wide variety of aquatic and terrestrial animals (Giesy and Kannan, 2001; Houde et al., 2006b; Kannan et al., 2004, 2005; Olsen et al., 2005; Taniyasu et al., 2003). The highest concentration of PFCs have been measured in fish-eating, apex predators, such as mink, bald eagles and aquatic mammals (Giesy and Kannan, 2001; Houde et al., 2005a). Some of the highest PFC levels reported in marine mammals are found in bottlenose dolphins inhabiting the estuarine waters of Charleston, South Carolina, an urban area of the southeast US (Houde et al., 2005b). Concentrations of PFCs found in the Charleston dolphins were on the same order of magnitude to that of occupationally exposed humans (Olsen et al., 2003a). Trophic biomagnification of PFCs was also reported in the dolphins' foodweb in CHS and in Sarasota Bay, Florida (Houde et al., 2006b).

Generally, there is a lack of clear trends in the relationship between age and accumulation of PFCs reported in the literature. Many studies in mammals have observed no correlation between PFCs and age (Dia et al., 2006; Kannan et al., 2002a,b, 2001; Van de Vijver et al., 2007). However, several reports have confirmed significantly higher PFC concentrations in pups and juvenile Antarctic elephant seals, harbor porpoise, dolphins and Baikal seals compared to adults (Houde et al., 2006a; Ishibashi et al., 2008a; Tao et al., 2006; Van de Vijver et al., 2003).

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PFCs frequently detected in biological samples are perfluoroalkyl sulfonates (PFSAs) and perfluoroalkyl carboxylates (PFCAs). The two PFCs used in the largest amounts in the US over the past 60 years are perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) (ASTDR, 2009). In 2000, 3 M Company voluntarily phased-out the production of perfluorooctyl sulfonyl fluoride (PFOSF) (Prevedouros et al., 2006) and since May 2009, PFOS and PFOSF have been included in Annex B (restricted) of the Stockholm Convention on persistent organic pollutants (POPs) (Stockholm Convention, 2009). As a result, significant drops in PFOS levels have occurred in some regions such as reported in Canadian Arctic ringed seals (Butt et al., 2007), sea otters along the California and Alaska coast (Hart et al., 2009; Kannan et al., 2006a) as well as in humans in the US (Olsen et al., 2008b). However, large reservoirs of PFOS and precursors in the environment, continuing use in products and continuing production in some countries (Paul et al., 2009; UNEP, 2007) contribute to persistence of these chemicals.

Because of the widespread environmental and human health concerns regarding PFC compounds, especially PFOS and PFOA, a large body of toxicological, epidemiological and environmental information has been published see reviews (Lau et al., 2004; Lau et al., 2007). Some PFCs have demonstrated developmental, reproductive, and carcinogenic toxicity in animals studies (Kennedy et al., 2004; Lau et al., 2007). PFCs are potentially harmful to marine mammals (Ishibashi et al., 2008b) and biochemical perturbations have been observed in wildlife species under field conditions as a consequence of exposure to PFOS (Hoff et al., 2004, 2005). Concern has been raised over the potential toxicity of persistent organic contaminants in marine mammals with a series of die-offs during the late 1980s and 1990s (Houde et al., 2005a; O'Shea, 1999; O'Shea and Tanabe, 2003). While the deaths that occurred during several of these epizootics were attributed primarily to morbillivirus, it was suggested that contaminants such as polychlorinated biphenyls (PCBs) and chlorinated pesticides may have been a contributing factor. A retrospective analysis of liver tissues from bottlenose dolphins that died during the high mortality epizootic along the Atlantic coast of the United States during 1987 and1988 found that concentrations of PFOS in the affected bottlenose were statistically greater than other species not affected during the epizootics, and to other bottlenose dolphin populations (Kuehl et al., 2009). Also in that study, PFOS concentrations in liver were found to be as great as, or greater than, concentrations of PCBs, chlorinated pesticides, and polybrominated diphenyl ethers (PBDEs).

Despite the ubiquitous occurrence of PFCs, very little is known regarding the impact of these contaminants on the health of wildlife populations. Exposure data is a critical component for assessing causal relationships between exposure and potential health effects and mitigating sources of exposure. The influence of biological variables such as age and sex are an important consideration in assessing contaminants and health-related data. The aim of this study was to compare the levels of PFCs in plasma of dolphins from two estuarine southeast US areas, Charleston, SC (CHS) and Indian River Lagoon (IRL), FL, for a three-year period (2003–2005) and to examine the influence of age, sex and location. This study extends the information on PFC concentrations in these dolphin populations reported by (Houde et al., 2005b) for 2003 and provides a greater sample size from which to investigate PFC concentrations as related to the above variables and to draw statistical conclusions.

2. Materials and methods

2.1. Study population

Samples were collected during bottlenose dolphin (*Tursiops truncatus*) capture–release health assessments conducted at two study sites, CHS and the IRL, during the summers of 2003–2005. Collections were conducted under NMFS Permit No. 998–1678, issued to Gregory Bossart, V.M.D., Ph.D. Detailed information pertaining to the study sites, methods for capture, sampling and release are described elsewhere (Fair et al., 2006). The CHS site (32°46′35″N, 79°55′51″W) included the Charleston Harbor, portions of the main channels and creeks of the Ashley, Cooper, and Wando Rivers, and the Stono River Estuary. For the IRL site, assessments were conducted near Titusville, FL (28°36′43″N, 80°48′27″W) and Stuart, FL (27°11′51″N, 80°15′10″W) and included portions of the Mosquito Lagoon, Indian River, Banana River, north and south forks of the St. Lucie River, and Sebastian Inlet. This study was part of the Bottlenose Dolphin Health and Risk Assessment (HERA) Project, aimed at assessing the health status of dolphins in these two areas and investigating associations between dolphin health and environmental stressors (Fair et al., 2006). Information pertaining to the study sites, methods for capture, sampling and release are described elsewhere (Fair et al., 2006). Age was determined by examining the post-natal dentine layers of an extracted tooth (Hohn et al., 1989). We evaluated PFC concentration in blood plasma samples collected from a total of 76 dolphins from CHS and 81 dolphins in the IRL.

2.2. Exposure assessment

Concentrations of PFCs in blood plasma were determined at the Environment Canada's Laboratories in Burlington Ontario. Sample extraction, analysis, and quality control procedures are detailed by Houde et al. (2005b). PFCs were quantified using high-performance liquid chromatography with negative electrospray tandem mass spectrometry (HPLC–MS/MS). PFC analytes determined are listed in Supplementary Table 1. Data quality assurance and control measures included both field and laboratory blanks, matrix spikes and standard material injection. Nondetect concentrations were replaced with ½ Minimum Detection Limit (MDL) for calculation of means.

2.3. Data analysis

Descriptive statistics for PFC concentrations presented in Table 1 includes the number of individuals, geometric mean, range, and 95% confidence intervals stratified by site, age, and sex. To meet assumptions of normality and homogeneity, concentrations of contaminants were log transformed. Significant differences between $\sum$PFCs, $\sum$PFCAs (PFOA, PFNA, PFDA, PFUA, PFDoA, PFTriA, PFTA, PFPA, see Supplementary Table 1 for acronym description), $\sum$PFSAs (PFOS, PFOSA, PFHxS), means in sex, age class, and site were assessed by analysis of variance (ANOVA) for greater than two categories and student's *t*-test for comparison of two categories using SAS (Version 9, SAS Institute Inc., Cary, NC). Additionally, the above PFC analytes were examined temporally for differences between the collection years (2003, 2004 and 2005) using ANOVA and post hoc comparisons. Interpretation of statistical significance testing should consider the small population sizes as a result of stratification by age, sex, and site. Sexual maturity in bottlenose dolphins has been categorized from 5 to 12 years for females and 10 to 13 years for males (Mead and Potter, 1990). In our study, adults were defined as females age 7 and older and males age 10 and older and juveniles categorized as less than these ages. Classification of the age/sex categories were juveniles (JUV), adult female (AF), and adult male (AM). Student's *t*-test comparison of PFC contaminants in juvenile males and females found no differences between these two groups, therefore, these were combined into one category termed 'juveniles' for a more robust comparison.

The relationships between the concentrations of different PFCs were examined using Pearson rank correlation. The relationship between $\sum$PFC and PFOS concentrations and age for CHS and IRL

Table 1
Summary of geometric mean PFC concentrations (ng/g wet weight), range, and 95% confidence limits in plasma from dolphins sampled during 2003–2005 in Charleston, SC (CHS) (n = 76) and the Indian River Lagoon, FL (IRL) (n = 81).

Parameter		Indian River Lagoon, FL			Charleston, SC		
		JUV	AF	AM	JUV	AF	AM
n		26	18	37	26	18	32
∑PFCs	Mean	**948**	**523**	**665**	**2340**[d,e]	**1330**[e]	**1570**[e]
	Min–max	113–3530	86.5–1640	112–4470	596–8670	587–3820	574.0–3630
	95% CI	670–1340	353–774	513–862	1800–3050	994–1770	1340–1840
∑carboxylic acids	Mean	**61.9**	**40.3**	**48.1**	**526**[d,e]	**265**[d]	**320**[e]
	Min–max	11.7–53.5	13.6–151	15.9–96.8	135–2010	73.7–601	91.6–1210
	95% CI	45.2–84.8	30.8–52.7	40.9–56.6	404–685	192–367	264–387
∑sulfonic acids	Mean	**848**	**478**	**607**	**1760**[d,e]	**1039**[d]	**1220**[e]
	Min–max	101–3450	72.0–1490	15.9–96.5	356–6790	513–3130	419–2640
	95% CI	615–1240	318–718	464.2–794	1330–2330	776–1390	1040–1430
PFDA	Geomean	15.6	12.8	12.7	212[d,e]	122[d]	130[e]
	Min–max	2.50–138	4.00–44.7	5.50–31.4	63.4–667	40.7–309	48.1–368
	95% CI	10.9–22.3	9.60–16.9	10.7–15.2	162–277	90.3–164	108–156
PFDS	Geomean	5.7	-	15.8	46.9	29.6	23.6
	Min–max	4.40–23.0	-	3.2–51.0	10.0–267	12.0–101	11.0–50.4
	95% CI	5.60–21.5	-	8.9–27.8	17.7–124	14.6–59.9	13.2–42.4
PFDoA	Geomean	1	1.2	1	10.5	6.2	7.5
	Min–max	0.30–14.0	0.40–3.60	0.20–5.30	0.30–62.4	0.50–31.7	0.30–136
	95% CI	0.60–1.70	0.90–1.80	0.80–1.40	5.90–18.7	3.60–10.8	5.00–11.0
PFHpA	Geomean	1.4	-	1.1	6.3	1.4	3
	Min–max	0.80–26.6	-	0.80–95.2	0.80–70.7	0.80–15.6	0.80–95.2
	95% CI	0.30–6.50	-	0.60–2.3	1.40–27.9	0.50–4.1	0.60–14.8
PFHxS	Geomean	75.6[b]	18.3[b]	42.8	78.4[d,e]	22.0[d]	40.2[e]
	Min–max	7.90–590	2.30–142	4.70–757	10.4–471	4.60–157	7.60–126
	95% CI	48.0–119	9.00–37.2	29.0–63.1	53.8–115	11.9–40.6	31.2–51.6
PFNA	Geomean	13.2b	5.70[a,b]	11.4[a]	108[d,e]	47.4[d]	63.2[e]
	Min–max	2.20–65.7	0.30–22.5	4.2–34.4	31.7–560	11.2–187	18.7–275
	95% CI	9.70–18.1	3.70–9.1	9.6–13.4	81.5–142	31.5–725	52.2–76.4
PFOA	Geomean	12.5[b]	3.90[b]	8.2	64.7[d,e]	15.1[c,d]	34.9[c,e]
PFOS	Min–max	2.00–70.3	0.30–33.9	1.90–39.9	3.40–560	0.30–99.9	8.00–275
	95% CI	8.40–18.7	2.10–7.40	6.40–10.6	44.4–94.2	7.80–29.4	27.2–44.6
	Geomean	787.5	453	553	1620[d,e]	979[d]	1140[e]
	Min–max	92.8–2850	69.2–1360	91.3–3620	316.7–6260	491–2910	395–2460
	95% CI	558.2–111.0	304.7–672.2	427–716	1222–2143	735–1300	973–1330
PFOSA	Geomean	1.5	1	1.2	41.3[e]	27.4	25.4[e]
	Min–max	0.50–1.15	0.50–6.50	0.50–15.2	10.7–124	7.50–66.5	7.70–69.3
	95% CI	1.00–1.10	0.60–1.50	0.90–1.60	32.1–53.2	20.8–36.1	20.9–30.7
PFTA	Geomean	0.8	0.4	0.7	0.8	1.5	0.9
	Min–max	0.40–1.70	0.40–0.90	0.4–1.70	0.4–3.30	0.40–1.70	0.40–19.8
	95% CI	0.50–1.10	0.30–0.50	0.60–1.00	0.50–1.20	0.90–2.20	0.60–1.30
	Geomean	0.6	0.5	0.5	1.6	-	2.1
PFTriA	Min–max	0.40–4.90	0.40–1.60	0.40–3.00	0.40–9.50	-	0.40–4.06
	95% CI	0.30–1.00	0.40–0.60	0.40–0.60	0.80–3.15	-	1.00–4.20
PFUA	Geomean	13.6	11.4	9.7	96.8[e]	57.1	64.1[e]
	Min–Max	2.60–73.8	2.40–48.0	1.80–62.7	21.9–496	14.5–220	10.2–583
	95% CI	9.80–19.1	8.30–15.8	8.10–11.7	70.3–133	39.3–83.0	50.5–81.3
PFOSA/PFOS	Mean	0.003	0.003	0.003	0.035	0.03	0.022
	SD	0.001	0.002	0.002	0.02	0.01	0.009

∑PFCs include PFDA, PFDS, PFDoA, PFHpA, PFHxS, PFNA, PFOA, PFOS, PFOSA, PFTA, PFTriA, and PFUA.
Statistical significance ($p < 0.05$). The following PFCs were found to be significantly different ($p < 0.05$) among similar dolphin age/sex groups between the two sites, CHS and IRL: ∑PFC, ∑PFCA, ∑PFSA, PFDA, PFDoA, PFNA, PFOA, PFOSA and PFUA [PFDS significant only among JUV, PFTA significant only among AF, PFTriA significant only among JUV and AM].

[a] Indicates significant difference between adult females (AF) and adult males (AM) in IRL.
[b] Indicates significant difference between juvenile males and females (JUV) and AF in IRL; indicates significant difference between JUV and AM in IRL.
[c] AF and AM in CHS.
[d] Indicates significant difference between JUV and AF in CHS.
[e] Indicates significant difference between JUV and AM in CHS.

dolphins was evaluated using an Analysis of Covariance (ANCOVA) model defined as *PFC = Location + Sex + Age*. Each model included terms to check for interaction between main effects (ANOVA) or between main effect and covariate (ANCOVA). We also examined length and weight since these variables are related to age.

3. Results and discussion

3.1. PFC concentrations in CHS and IRL dolphins

Geometric means of ∑PFCs, ∑PFCAs, ∑PFSAs in plasma were significantly higher in CHS dolphins for all age/sex categories compared to IRL dolphins (Table 2). PFCs were consistently higher in CHS dolphins and plasma ∑PFCs geometric means were highest in CHS dolphins for all age/sex categories compared to IRL dolphins by a factor of 2–3. Similarly, CHS dolphins had significantly greater levels of ∑PFCAs and ∑PFSAs ranging from 2 to 9 times higher among the different age classes. In addition to total PFCs and classes, between site differences were also noted for the following individual compounds with significantly higher concentrations occurring in the CHS dolphins in their respective age/sex class: PFDA, PFDS, PFDoA, PHHxS, PFNA, PFOA, PFOS, PFOSA, and PFUnA. The only two analytes that were not significantly different between the dolphin groups from the two sites were PFHxS and PFNA (Table 1).

Table 2
Comparison of plasma concentrations (ng/g wet weight) of ∑PFC, ∑PFCA, ∑PFSA and individual compounds from Charleston, SC and Indian River Lagoon, FL dolphins for 2003, 2004 and 2005.

	Age	∑PFC	∑PFCA	∑PFSA	PFDA	PFDS	PFDoA	PFHpA	PFHxS	PFNA	PFOA	PFOS	PFOSA	PFTA	PFTriA	PFUnA
CHS 2003	*n* = 46															
Geomean	15	1570[a]	288[a]	1260[a]	132	15.3	8.22	0.83	33	51.5	33	1190	24.5	0.51	1.52	50.4
SD	7.9	890	237	686	109	0.68	13	0	35.5	42.3	36.2	652	20	0.55	1.36	61.7
Max	33	4520	1250	3270	542	15.8	62.4	0.83	164	214	163	3070	102	1.74	4.11	320
Min	3	586	73.7	513	40.7	14.9	0.5	0.83	4.64	11.2	4.7	492	7.5	0.4	0.4	10.2
CHS 2004	*n* = 19															
Geomean	12	1250[a]	360[a]	881[a,b]	132	0	10	0	31.7	66.72	34.6	813	27.4	0.63	2.04	95
SD	8.2	951	349	623	114	0	36.2	0	69.4	51.3	25.1	571	16.4	1.34	5.72	159
Max	28	3700	1320	2420	483	0	136	0	321	278	123	2310	67.6	4.06	19.8	583
Min	3.5	574	155	356	59.3	0	1.5	0	5.88	29.2	11.2	317	8.82	0.4	0.4	23.9
CHS 2005	*n* = 17															
Geomean	13	2450[b]	493[a]	1860[b]	180	36.5	5	3.57	83.3	131	33.5	1710	46	1.8	0	104
SD	8.4	2150	530	1660	182	68.2	10.2	27.1	133	141	148	1520	29	0.12	0	116
Max	24	8670	2010	6790	667	267	30.1	95.2	471	561	561	6260	123	2.25	0	496
Min	2.5	696	135	524	55	10	0.25	0.76	8.46	28	0.25	491	16.7	1.74	0	25.3
IRL 2003	*n* = 38															
Geomean	13	573[a]	50.8[a]	516[a]	14.8	0	1.55	0	30.8	11.1	6.83	473	1	0.4	0.4	10.9
SD	6.3	691	46.5	658	11.8	0	1.68	0	1	9.66	16.8	568	1.76	0	0	16.2
Max	26	2390	227	2297	53.2	0	6.07	0	332	50.9	70.3	2010	6.51	0.4	0.4	64.1
Min	3.5	86.5	14.5	72.02	4.4	0	0.5	0	2.31	3.22	0.5	69.2	0.5	0.4	0.4	1.78
IRL 2004	*n* = 30															
Geomean	11	607[a]	42.4[a]	561[a]	11.54	0	1.22	0	34.4	7.09	7.33	520	0.94	0.45	0.55	10.5
SD	4.2	565	38.6	541	13.4	0	2.34	0	64.2	5.94	9.12	480	1.1	0.26	0.97	12.3
Max	19	2190	228	2100	78.7	0	12.86	0	227	34.1	38.13	1870	4.59	1.57	4.91	73.8
Min	3.5	113	11.7	101	2.48	0	0.4	0	2.6	0.25	0.25	92.8	0.45	0.4	0.4	2.58
IRL 2005	*n* = 12															
Geomean	10	1750[a]	70[a]	1640[a]	18.9	14.6	0.43	1.1	147	18.9	11.2	1450	4.56	1.74	0	13.5
SD	5.5	1470	85.8	1430	35.7	14.5	4.03	7.4	283	15.4	19.1	1180	4.89	0	0	16.9
Max	23	4470	353	4380	138	51	14	26.6	757	65.6	65.6	3620	15.2	1.74	0	68.4
Min	4.5	361	31.1	320	8.14	3.17	0.18	0.83	16.9	8.75	2.11	303	0.93	1.74	0	7.34

a,b,c = different superscripts indicates significant difference ($p < 0.01$) between mean concentrations ∑PFCs, ∑PFCAs, ∑PFSAs; note age is arithmetic mean.

The ∑PFCAs were much greater in CHS dolphins (22%) compared to IRL dolphins (7%) while the ∑PFSAs were 79% in CHS dolphins versus 93% in IRL dolphins (Fig. 1a). CHS and IRL dolphins clearly have different distributions in these two classes of PFCs. The compositional profile of individual PFCs differed by site as well (Fig. 1b). Of all the PFCs, PFOS accounted for the main difference between the two sites with much higher levels in IRL dolphins (~84%) compared to CHS dolphins (~73%). All of the remaining PFCs were higher in CHS dolphins with PFDA accounting for the highest percentage difference (9% CHS; 2% IRL). The observed variations in patterns could be due to differences in environmental exposures, diets or biotransformation metabolism. In both dolphin populations, PFOS was the major contributor to total PFC which is similar to that found in studies with marine mammals and other aquatic biota (Houde et al., 2011, 2005a).

The PFOSA/PFOS ratio was also examined since PFOSA is a precursor of PFOS (Table 1). Interestingly, PFOSA was ~25 times higher in CHS dolphins and the PFOSA/PFOS ratio for all age classes of CHS dolphins was 0.027 (±0.014 SD) compared to approximately 0.003 (±0.002 SD) for IRL dolphins. The PFOSA:PFOS in CHS dolphins represents a magnitude of ten times higher than IRL dolphins and suggests a greater prevalence of PFOS and PFOS precursors in their environment. PFCs have been shown to bioaccumulate in food webs (Houde et al., 2006b). As top-level predators, dolphins

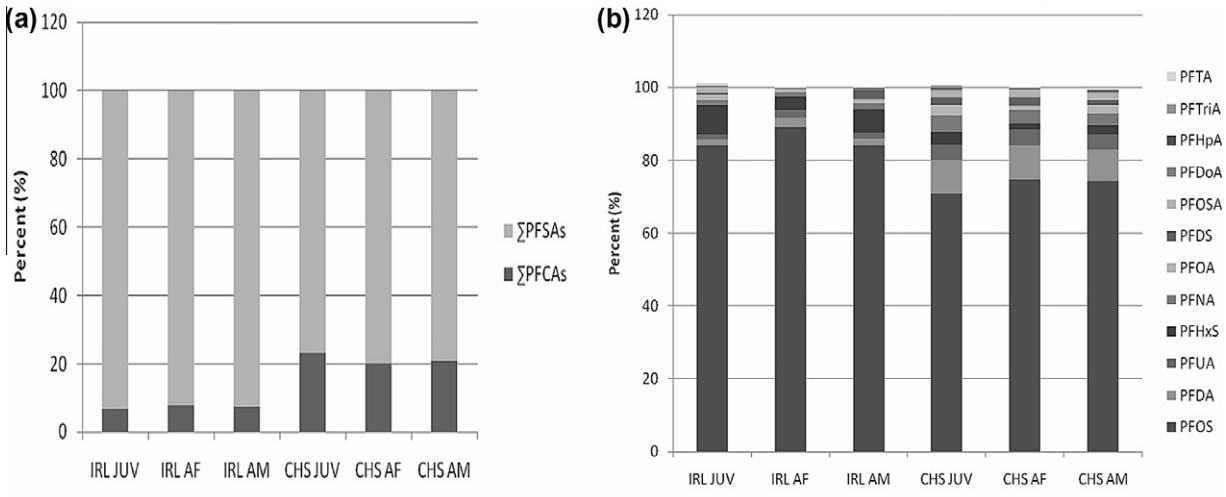

Fig. 1. Plasma PFCs found in juvenile (JUV) and adult male (AM) and female (AF) dolphins from Charleston, SC (CHS) and the Indian River Lagoon (IRL), FL (a) mean composition percentage of total PFSAs and PFCAs and (b) mean composition profile of individual PFCs.

serve as sentinel species (Bossart, 2010) and may reflect the PFC inputs in their environment.

Adult and juvenile dolphins in CHS had significantly higher levels of all total PFC comparisons (∑PFCs, ∑PFCAs, ∑PFSAs) as well as the majority of the individual compounds (Table 1) compared to IRL dolphins. Geographical differences exist in temporal trends of PFC contamination (Ishibashi et al., 2008a; Kannan et al., 2001) and habitat and diet are prominent factors that influence PFC concentrations in marine mammals (Houde et al., 2011; Moon et al., 2010). Non-point source pollution originating from urbanized sites was reported in aquatic ecosystems as a key factor in PFC contamination (Kim and Kannan, 2007; Zuchi et al., 2008). The fact that CHS dolphins had higher PFC concentration than IRL dolphins is not surprising, as CHS is more urbanized, although point sources may also be a contributing factor to the high PFC levels in dolphins from this area. Even within the CHS study site, fine-scale spatial variation of PFCs was found with higher body burdens of specific PFC compounds in dolphins inhabiting areas with greater developed land use (Adams et al., 2008). Thus, localized releases of PFCs are potential sources of PFC exposure for these dolphins. The differences observed in PFC patterns in dolphins from these two sites are likely due to sources of local inputs and/or transport processes for these two locations. Future studies should focus on source tracking of PFCs in the dolphins' environment, particularly in CHS where extremely high PFC levels were found.

Temporal differences in ∑PFC, ∑PFCA and ∑PFSA concentrations occurred in dolphins from both sites (Table 3). Both CHS and IRL dolphins had significantly higher ∑PFC and ∑PFSA levels in 2005 compared to 2003 and 2004. Only CHS dolphins had significantly higher levels of ∑PFCA in year 2005 vs. 2003. The mean ages were similar for the dolphins at both sites for each of the years (CHS = 15, 12 and 13 years; IRL = 13, 11 and 10 years). In order to examine temporal trends a longer time series would be needed, although these preliminary findings should be further investigated as PFCs have been shown to impact survival, growth and hormone/enzyme function in animal models (Seacat et al., 2003; Kennedy et al., 2004).

Pearson correlation analysis showed that the concentrations of PFCs were significantly correlated with each other ($r = 0.25–0.88$) with the exception of PFDoA and PFHxS, PFNA, PFOS, PFOSA in IRL dolphins. Highly significant correlations were found between PFCA and PFSA concentrations. Correlations between these two classes (e.g., PFCA and PFSA) are of interest as they cannot convert directly into each other (Kannan et al., 2004; Olsen et al., 2003b) and provide insight into potential sources of exposure. The correlation between PFOA: PFOS for IRL (0.73) and CHS (0.46) dolphins may indicate different sources of exposure to these two PFC classes. Associations between adjacent PFCAs within sites suggest similar exposure and common origins of compounds. Correlations between PFHxS:PFOS and PFOSA:PFOS were similar for CHS and IRL. PFOS is known to be a degradation product of PFOSA (Tomy et al., 2004).

3.2. Age and sex-related differences in PFC concentrations

Overall, plasma ∑PFCs geometric means were highest in juvenile dolphins from the CHS site (2340 ng/g w.w.) and these were significantly higher than concentrations found in either adult females or adult males from this site (Table 1). Similar results were observed for ∑PFCAs, ∑PFSAs, PFDA, PFHxS, PFNA, PFOA, and PFOS. Significantly higher levels of PFTA and PFUA also occurred in juveniles but this comparison was observed only for adult males. Adult males in CHS had significantly higher levels of ∑PFCs, ∑PFCAs, ∑PFSAs compared to females, in addition to several individual compounds including PFDA, PFHxS, PFNA and PFOS. For the IRL site, no statistical differences were found among any of the age/sex classes in the geometric means of ∑PFCs, ∑PFCAs, ∑PFSAs, and although the concentrations for juveniles were higher than those for adults, these differences were not statistically different. Only limited differences were observed in any of the individual analytes in IRL dolphins with PFNA significantly higher in juveniles and adult males compared to adult females, and PFHxS and PFOA were significantly greater in juveniles compared to adult females. The dominant PFC compound was PFOS in all age groups for both sites, averaging 72% (69% juveniles; 74% adult females; 73% adult males) in CHS dolphins and 84% (83% juveniles; 87% adult females; 83% adult males) in IRL dolphins.

Many marine mammal studies have reported sex-specific differences in concentrations of persistent organohalogen compounds such as PCBs, DDT and PBDEs, and since females offload contaminants to their young they generally contain lesser concentrations than those of males which tend to increase with age (Borrell et al., 1995; Cockcroft et al., 1989; Fair et al., 2010, 2007; Krahn et al., 2009). However, only a few studies have reported sex-related differences in PFC levels in marine mammals (Ahrens et al., 2009b; Van de Vijver et al., 2007). In this study, differences were found only in CHS dolphins with adult males having significantly higher ∑PFC levels compared to adult females, which were not observed

Table 3
Pearson correlation coefficients[a] among log-transformed concentrations of the major PFCs found in plasma of dolphins from (a) Charleston, SC and (b) the Indian River Lagoon, FL.

	PFDA	PFDoA	PFHxS	PFNA	PFOA	PFOS	PFOSA	PFUnA
(a) Charleston								
PFDA	1.00							
PFDoA	0.76							
PFHxS	0.63	0.32						
PFNA	0.77	0.40	0.71					
PFOA	0.53	0.39	0.52	0.67				
PFOS	0.83	0.55	0.75	0.69	0.46			
PFOSA	0.71	0.39	0.59	0.73	0.47	0.69		
PFUnA	0.79	0.64	0.57	0.72	0.45	0.61	0.63	1.00
(b) Indian River Lagoon								
PFDA	1.00							
PFDoA	0.60							
PFHxS	0.40	0.06						
PFNA	0.56	0.15	0.55					
PFOA	0.56	0.25	0.85	0.61				
PFOS	0.54	0.15	0.88	0.48	0.73			
PFOSA	0.30	0.01	0.62	0.49	0.43	0.71		
PFUnA	0.61	0.42	0.50	0.48	0.47	0.67	0.56	1.00

[a] Correlations >0.2 are significant.

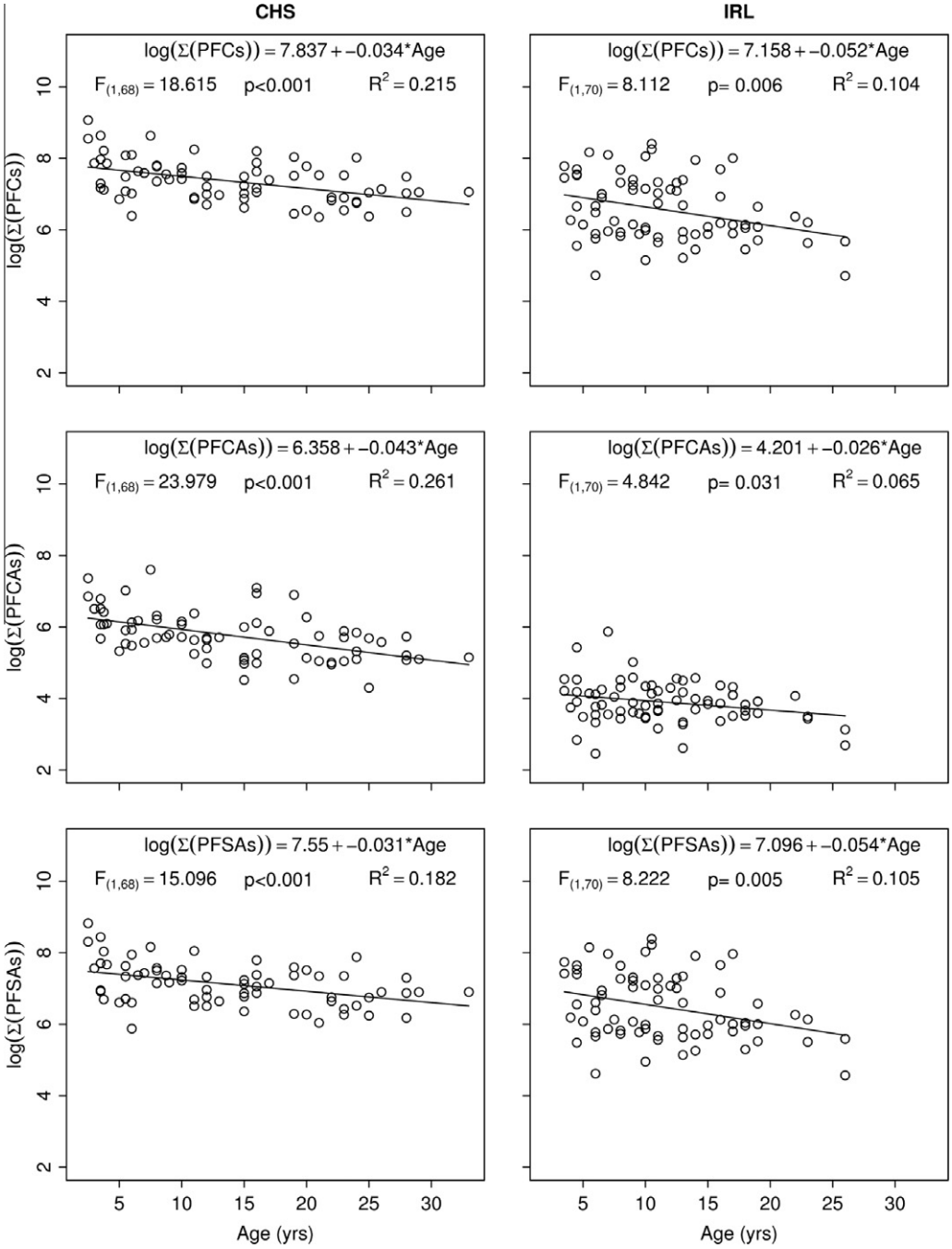

Fig. 2. Age-adjusted comparison of plasma concentrations of ∑PFC, ∑PFCA, and ∑PFSA in bottlenose dolphins (*Tursiops truncatus*) sampled from Charleston, SC (CHS) and the Indian River Lagoon, FL (IRL) during 2003–2005.

in the IRL dolphins. The lack of sex-related concentration patterns of PFCs in IRL dolphins as well as other marine mammals (Ishibashi et al., 2008b) reflects a different trend than typically observed in marine mammals for persistent organochlorine compounds. However, the high PFC levels in the CHS dolphins may have contributed to the manifestation of PFC accumulation differences between males and females.

Analysis of covariance (ANCOVA) found the main effects of location to be significant, effects of sex to be non-significant and age as covariated to be highly significant. Hence, the relationship of ∑PFC, ∑PFCA, and ∑PFSA concentrations with age was examined using linear regression for the two dolphin populations, separately, but with males and females combined (Fig. 2). Analysis of ∑PFC, ∑PFCA, and ∑PFSA, the dominant PFCs, in male and female dolphins from CHS and IRL revealed that levels decreased significantly with age (Fig. 2). The coefficient of determination (R^2) for ∑PFC, ∑PFCA, and ∑PFSA for CHS dolphins (0.22, 0.26, and 0.18) was higher than that found for IRL dolphins (0.10, 0.07, and 0.11), respectively, however, the relationship was weak. The plasma ∑PFC, ∑PFCA, and ∑PFSA concentration also decreased significantly with respect to size (i.e., using length and weight as independent variables, data not shown).

The effects of age on accumulation of PFC with higher levels occurring in young CHS and IRL dolphins for the three-year dataset (2003–2005) confirm results reported earlier based on data from 2003 (Houde et al., 2005a,b). This is also in agreement with several

other marine mammal studies. Juvenile sea otters, melon-headed whales, and seals were found to have higher levels of PFCs than adults (Ahrens et al., 2009b; Hart et al., 2008; Houde et al., 2006a; Ishibashi et al., 2008a). Juvenile Antarctic elephant seals, harbor porpoise, and Baikal seals also had higher levels compared to adults (Ishibashi et al., 2008a; Tao et al., 2006; Van de Vijver et al., 2003). While no correlations with age were found in the Sarasota Bay, Florida dolphin population (Houde et al., 2005a,b), higher PFC concentrations were observed in sexually immature bottlenose dolphin calves (<10 years) from Sarasota compared to their mothers (1410 ± 1780 ng/g vs. 366 ± 351 ng/g ww) (Houde et al., 2006a). In contrast, other studies in marine mammals have reported either no age-related PFC accumulation (Van de Vijver et al., 2007) while others have found significant increases occurring with age such as reported in polar bears (Smithwick et al., 2005). Harbor seals with bronchopneumonia were reported to have lower PFC concentrations than individuals without this disease (Van de Vijver et al., 2003). Pathological processes may also affect concentrations as PFOS levels in sea otters were higher in those that died from infectious disease than from other causes (Kannan et al., 2006b). It has been speculated that the presence of diseased animals may hamper the occurrence of age-related significant correlations due to interference in accumulation and metabolism of PFOS (Dorneles et al., 2008).

The high levels found in juvenile dolphins are likely a combination of PFCs transferred from the mother by milk (Houde et al., 2006a) and also from prey consumed during its early years. In humans, PFOS is readily transferred to the fetus through the placenta (Inoue et al., 2004; Midasch et al., 2007) and to the infant through breast feeding (Karman et al., 2007; So et al., 2006). The findings of this study, indicating higher levels of PFCs found in juvenile dolphins at both sites and particularly for CHS dolphins, suggest concern over the potential health impacts on neonate and calves. In 2000, the 3M Company, the primary global manufacturer of POSF, phased-out manufacturing of this compound, subsequent to the widespread detection of PFOS in humans and wildlife, although production continues in other countries at reduced volumes (Paul et al., 2009; UNEP, 2007) and existing stocks e.g. PFOS in aqueous film forming foam (AFFF), are still in use. Since then, concentrations in some US populations have been reported to decline (Olsen et al., 2008a). However, considering the long half-life of these chemicals and their persistence and recycling in the environment and studies that report effects at very low concentrations it is apparent that health concerns of PFCs will continue in both wildlife and humans.

4. Conclusion

The results from this study indicate that age, sex, and location affect circulating concentrations of PFCs in bottlenose dolphins. Marked differences occurred in PFC concentrations associated with age in both dolphin populations albeit elevated PFC occurred in CHS compared to the IRL. The accumulation of PFCs related to sex was only found in the highly exposed CHS population with levels higher in adult males compared to adult females. Limited information exists on the sources and environmental fate, toxicokinetics and effects of PFCs for marine species. Assessing exposure of PFCs in marine mammal populations may provide useful information in understanding the pathways of exposures to PFCs and potential sources. The resulting high levels in juvenile dolphins, especially those in CHS, occurring during a period when their biological systems are undergoing rapid growth and development may cause a greater risk for health and reproductive impacts. Evaluating the magnitude of exposure to PFCs in these dolphin populations, especially the CHS dolphins, is of interest for conservation purposes, especially since this class of pollutants have been shown to be endocrine disruptors, tumor promoters and immunosuppressors. Further studies are under way to determine whether PFC plasma levels are associated with health effects in these dolphin populations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.marpolbul.2011.10.022.

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